A TCR-pMHC confinement time model of T cell activation

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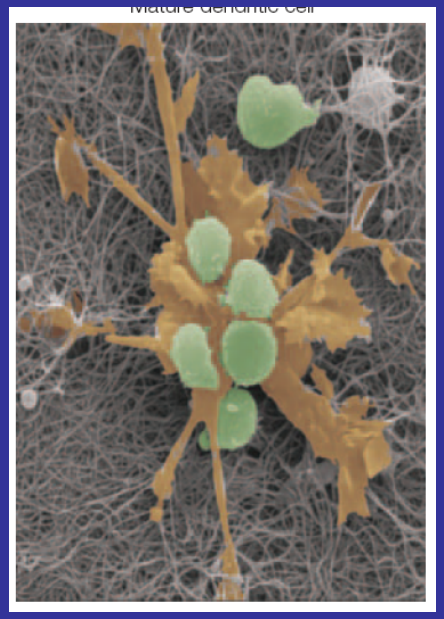
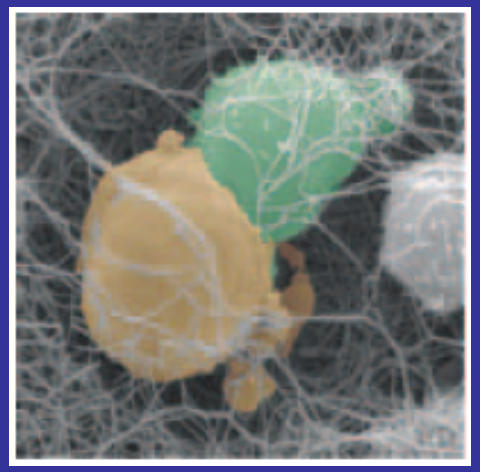
Understanding T cell activation is important

• T cells perform surveillance on antigen-presenting-cells (APCs), looking for signs of invaders

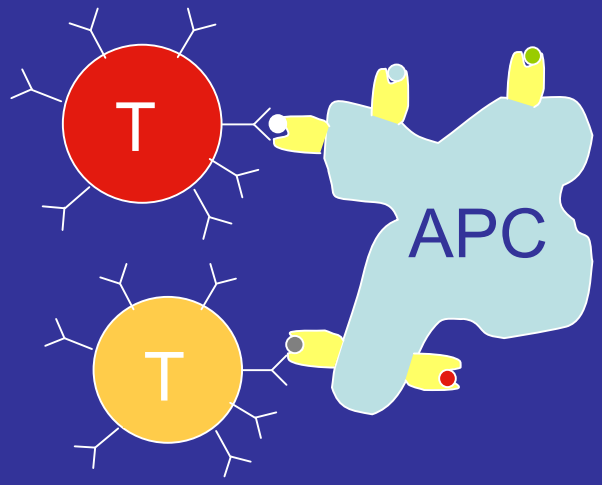
• Upon activation, they can
  • directly kill infected cells
  • activate B cells that secrete antibodies
  • activate macrophage cells





• T cell receptors bind to peptide-MHC complexes (pMHC) on antigen-presenting cells (APC)

Each T cell holds ~50,000 identical T cell receptors (TCR).



Specificity: TCR on a particular cell bind well to only a small class of pMHC

Sensitivity: Some T cells can be at least partially activated by (very) few pMHC.

Speed: If nothing is recognized, T cell moves on quickly.

TCR signal locally but most expts measure cellular responses:

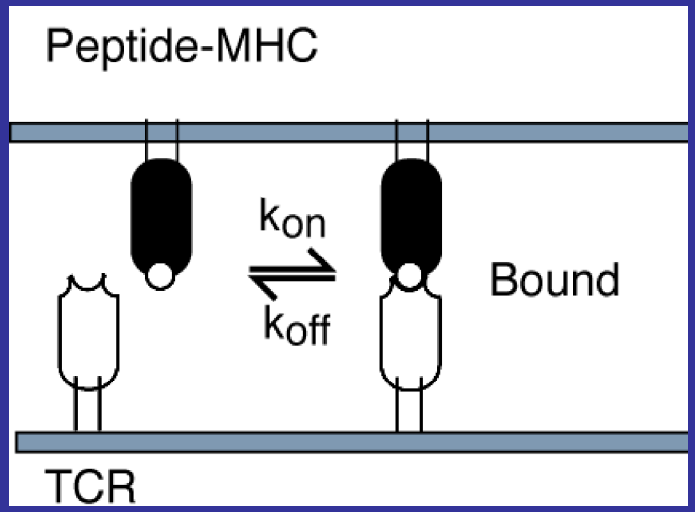
- Cytokine release
- TCR downregulation

Can we predict the T cell response based on the physical properties of the TCR-pMHC bond?

(the answer has to be YES)

1. Introduction
2. Measuring bond properties
3. Previous models of T cell activation:
   Serial engagement, kinetic proofreading, et al
4. Confinement time model of TCR signaling (2009)
5. Theoretical properties of confinement time models

Quantifying pMHC-TCR interaction

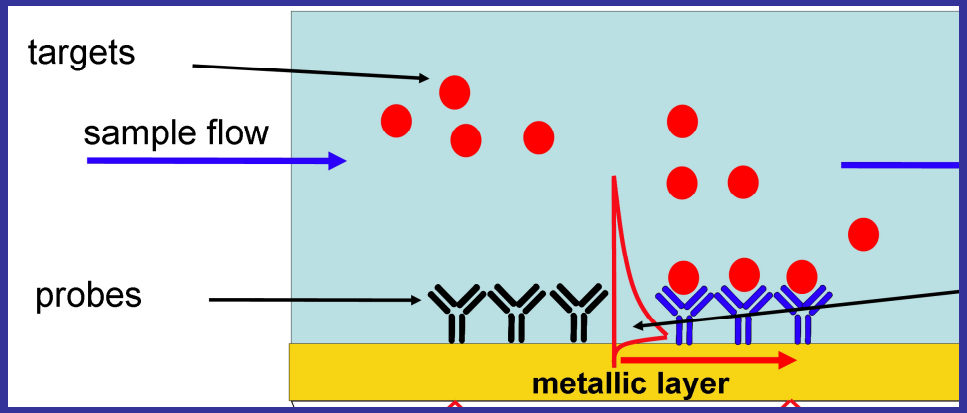


forward rate = k_{on}
reverse rate = k_{off}
half-life $t_{1/2}$ = ln(2) / k_{off}
Dissociation constant
= k_{off} / k_{on}
= K_D

- Measure on and off rates for the reaction using SPR (BIAcore).

- Bonds are found to be weak and transient.

Two and three dimensional kinetics

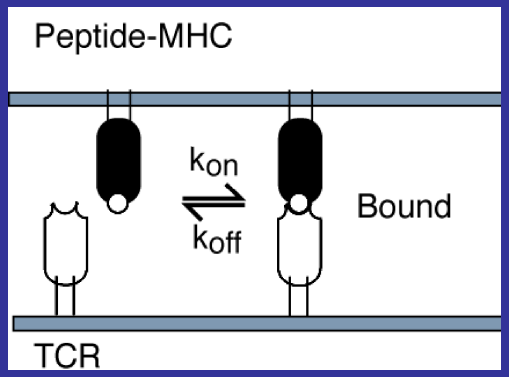


SPR measurements are 3-D:

$[k_{on}] = M^{-1}s^{-1} = cm^3\ s^{-1}$

$[k_{off}] = s^{-1}$

$[K_D] = M = cm^{-3}$



At the immune synapse:

2-D constrained kinetics

$[k_{on}] = cm^2\ s^{-1}$

$[k_{off}] = s^{-1}$

$[K_D] = cm^{-2}$

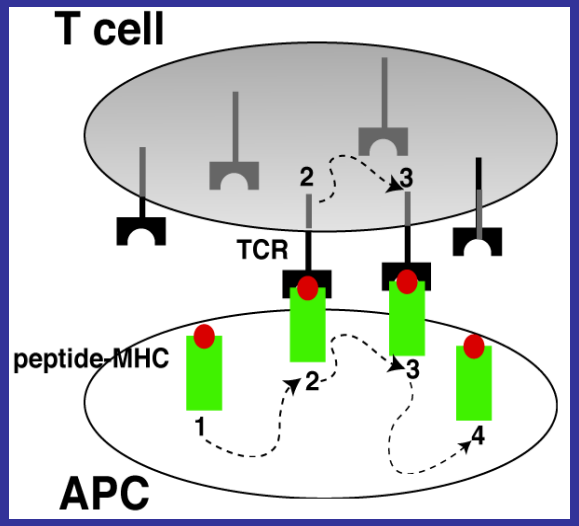
- Measurements of 2-D kinetics are rare!
- Popular heuristic (probably unreliable!):

  $k_{on}^{2D} = k_{on}^{3D} / L$ where L ~ 10nm

  $k_{off}^{2D} = k_{off}^{3D}$ (receptor scale)

- Also: mechanical forces on bonds at the synapse.

Serial Engagement Model

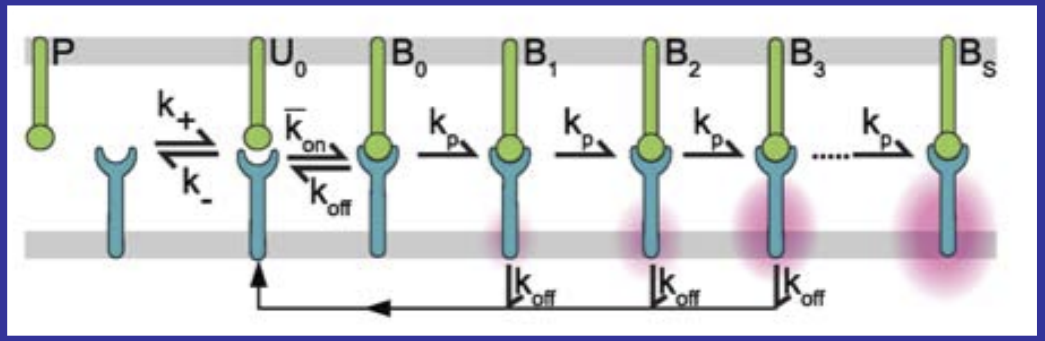


pMHC that bind TCR for a *short* time will bind the most TCR during their time in the contact region.
(high k_{off} is good)

Valitutti et al. (1995) Nature **376**:148.

Quantity ← – – – – – → **Quality**

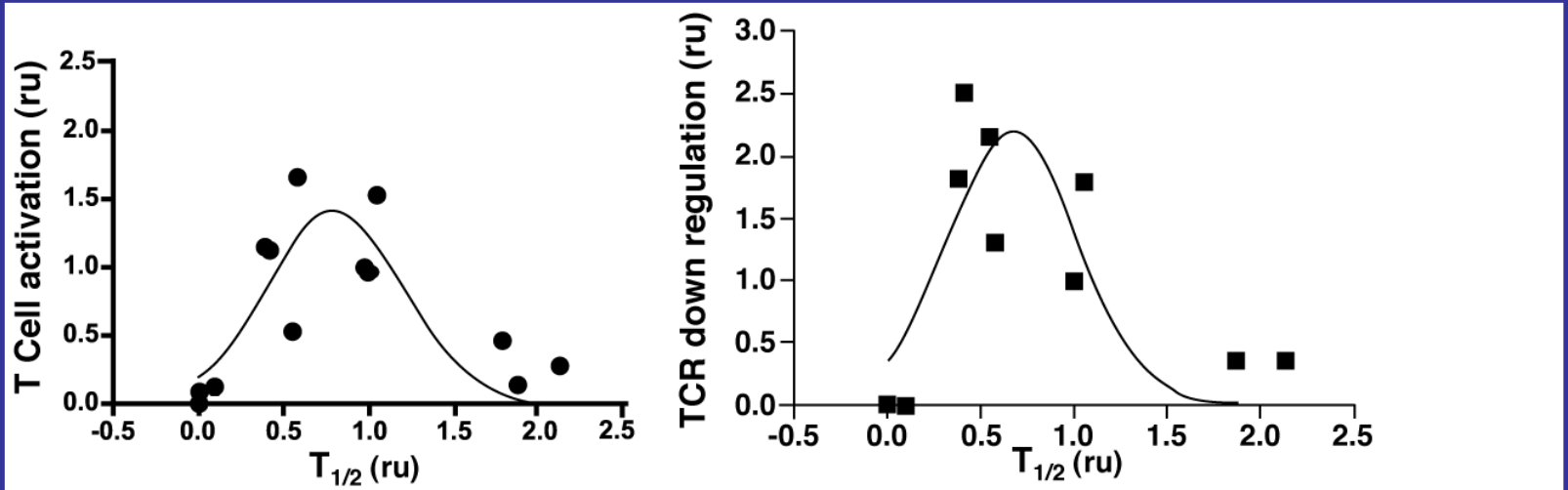
Kinetic Proofreading Model



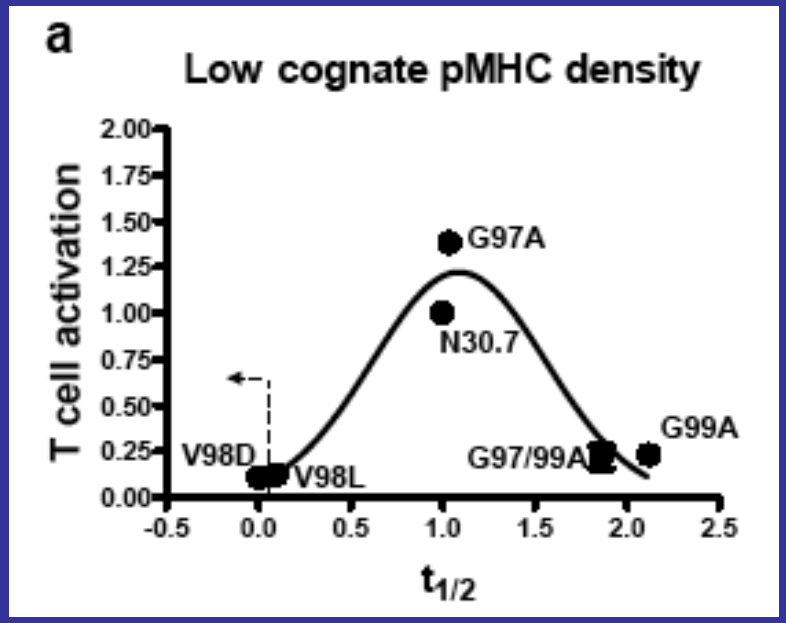
a series of biochemical events leading to full activation must occur before the TCR-pMHC bond breaks
(low k_{off} is good)

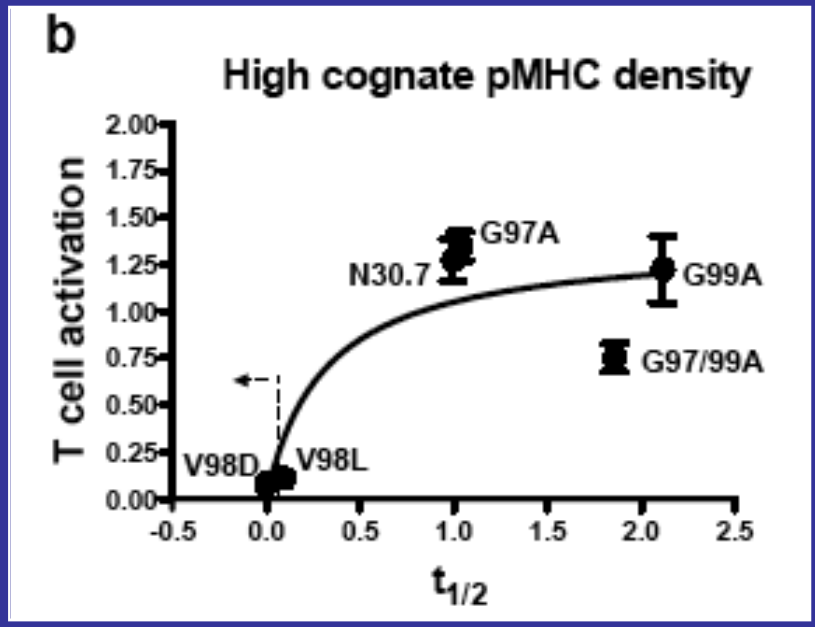
McKeithan 1995, PNAS **92**:5042

Experimental support for k_{off} models



Kalergis et al. (2001) *Nature Immunol.* **2**:229-234 Coombs et al. (2002) *Nature Immunol.* **3**:926-93



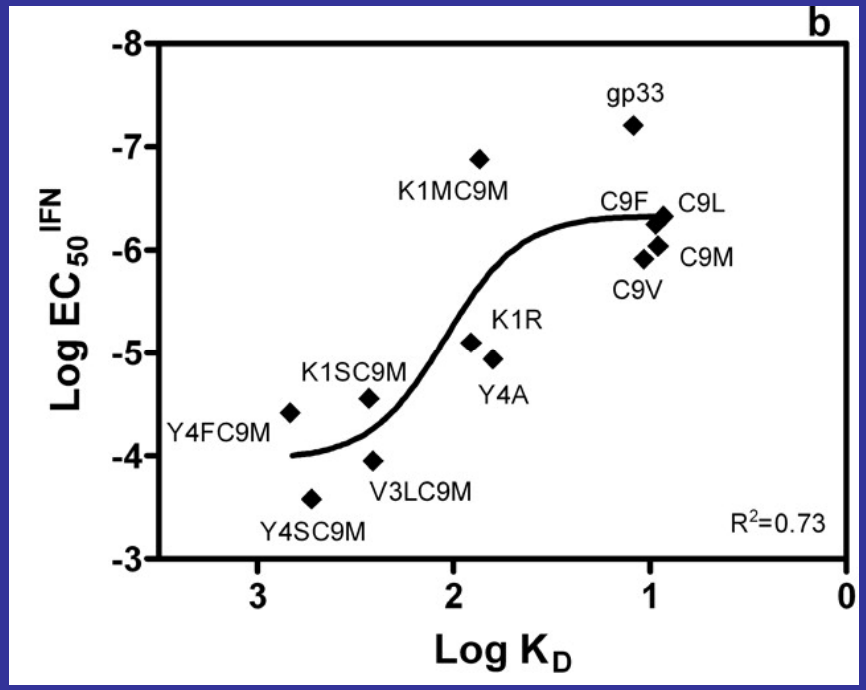


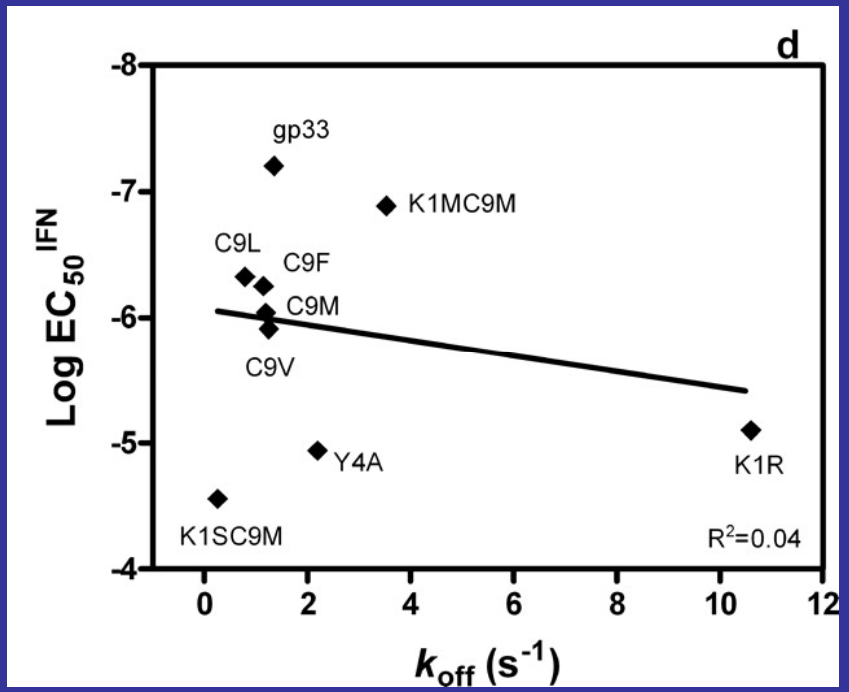
(Gonzalez et al PNAS 2005)

We focused on the role of the dissociation rate, $k_{off.}$ Other studies underlined the importance of the dissociation constant, K_D.

CD8$^+$ T Cell Activation Is Governed by TCR-Peptide/MHC Affinity, Not Dissociation Rate[1]

Shaomin Tian,* Robert Maile,*† Edward J. Collins,*‡ and Jeffrey A. Frelinger[2]*

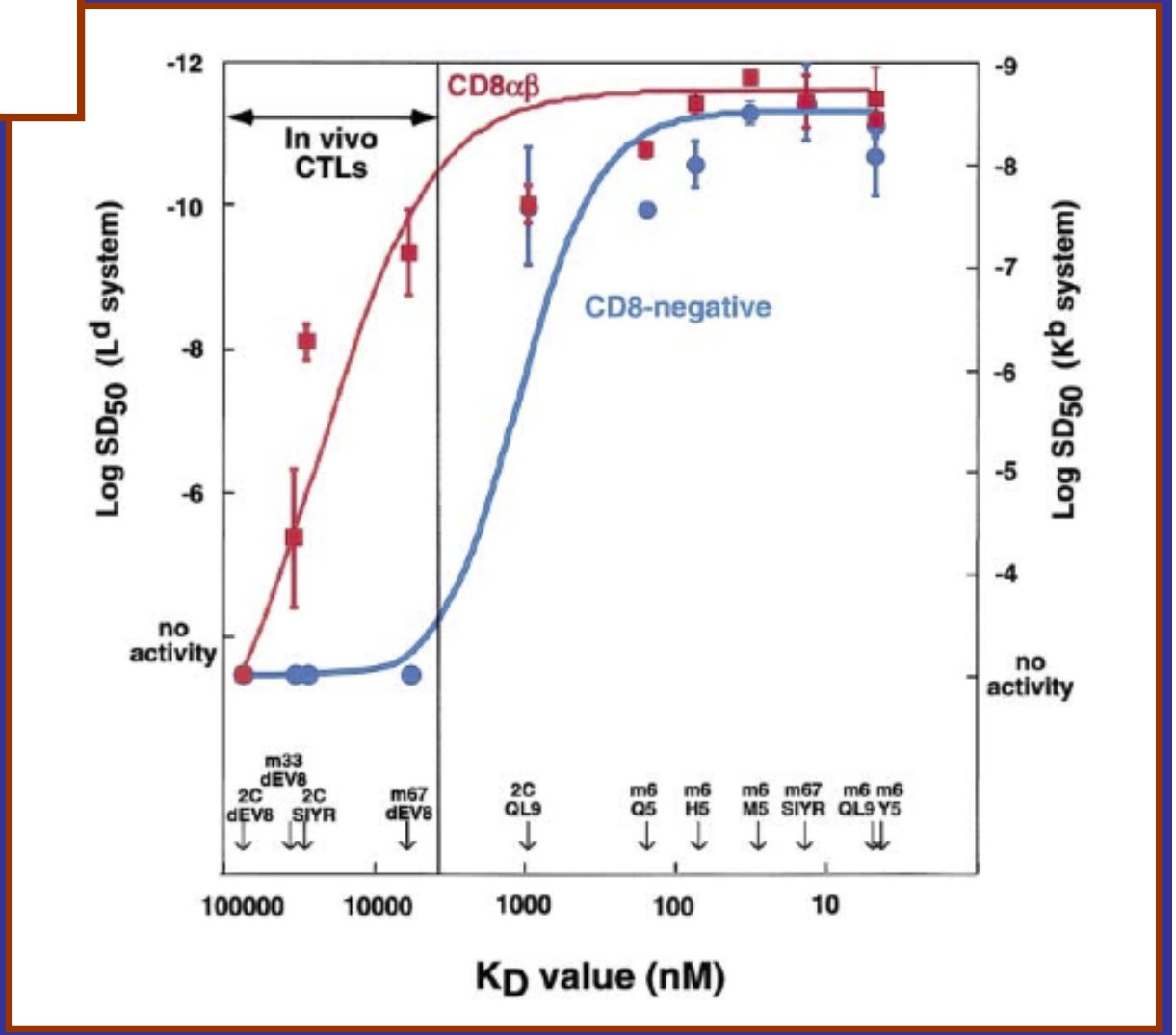




Immunity, Vol. 18, 255–264, February, 2003, Copyright ©2003 by Cell Press

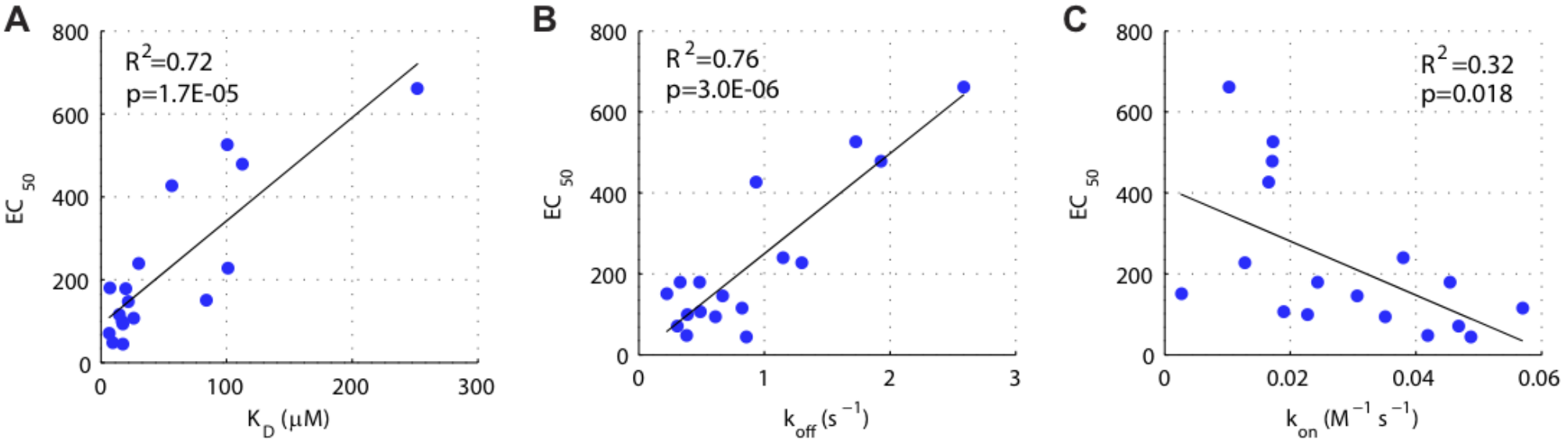
Quantitative Analysis of the Contribution of TCR/pepMHC Affinity and CD8 to T Cell Activation

Holler and Kranz, Immunity, 2003



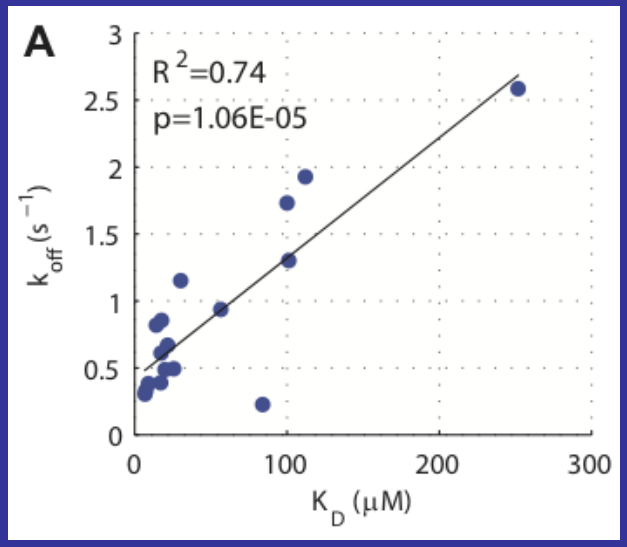
New experimental work: Aleksic, Dushek et al (Immunity, in press)

- detailed SPR study of 1G4 TCR binding to 17 different altered peptide ligands with wild-type or mutated MHC.
- analyzed the activation of CTL clones by plate-bound pMHC
- precise control for equal pMHC presentation on plates.

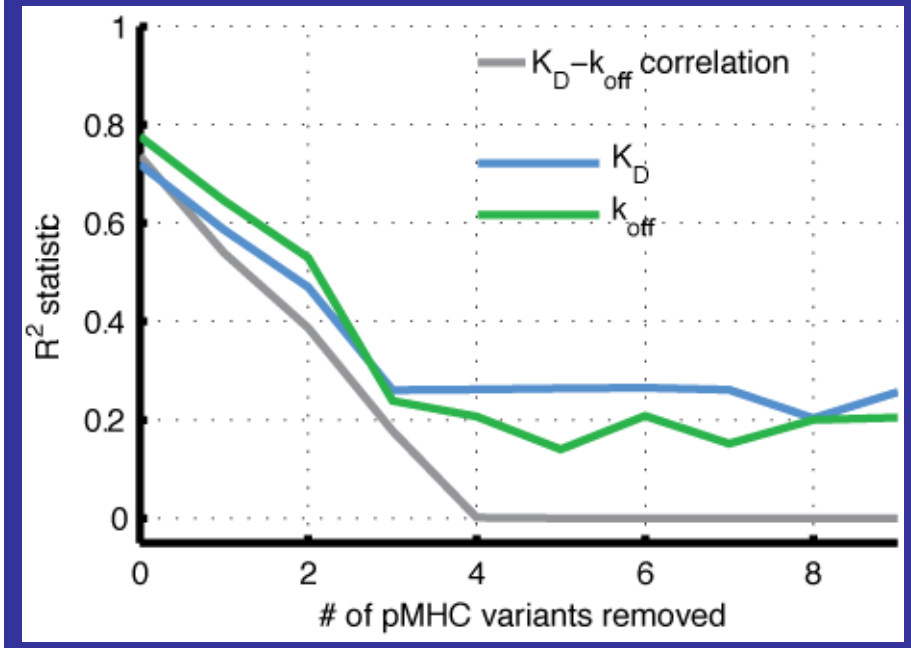


- However, there is a confounding correlation between K_D and k_{off}!

Subset analysis and bias

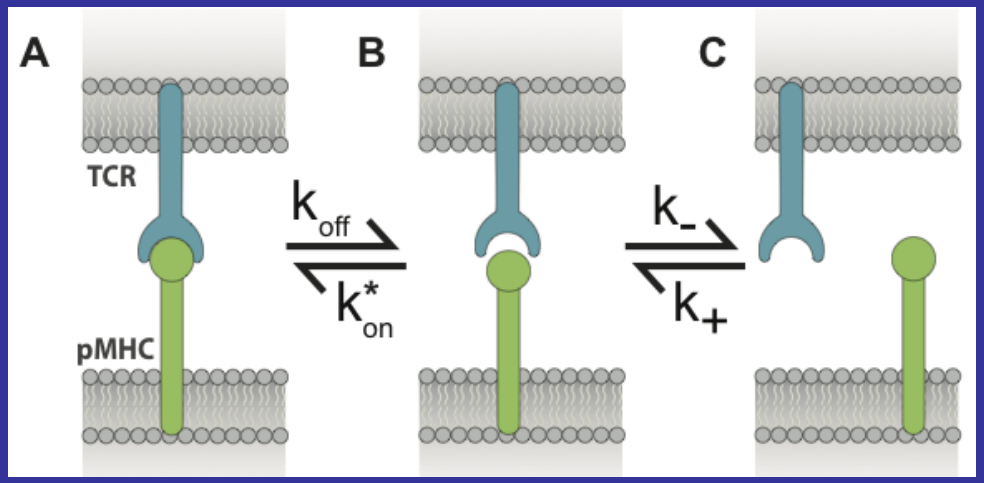


- The correlation between K_D and k_{off} is large.



- We extracted subsets that maximize variation in k_{on}.
- K_D and k_{off} fit poorly as subset shrinks.
- Once three pMHC variants are removed, K_D and k_{off} do not correlate significantly with pMHC potency ($p>0.05$)

A new model for surface receptor binding



Membrane diffusion is slow and therefore reactions are likely to be diffusion-limited.

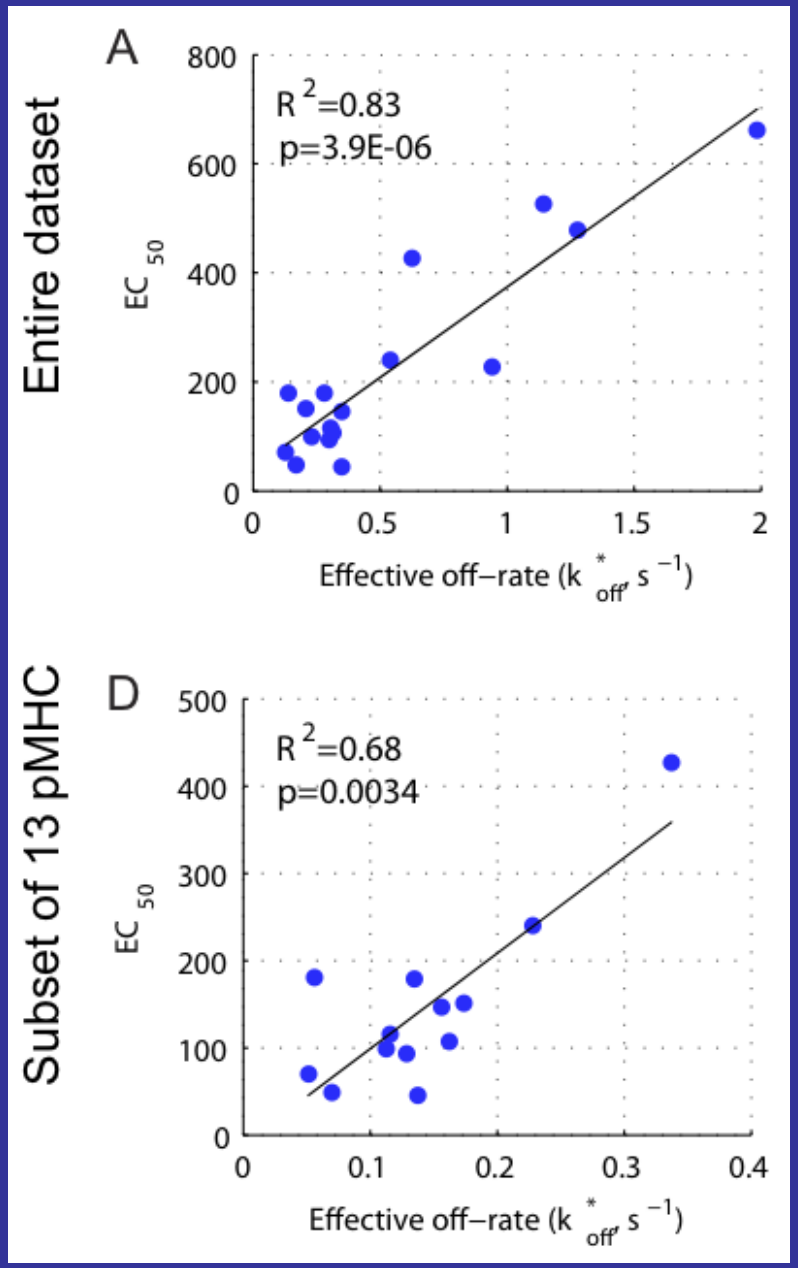
This aspect is missing from some existing models.

$$k^*_{off} = k_{off}\left(\frac{k_-}{k^*_{on} + k_-}\right)$$

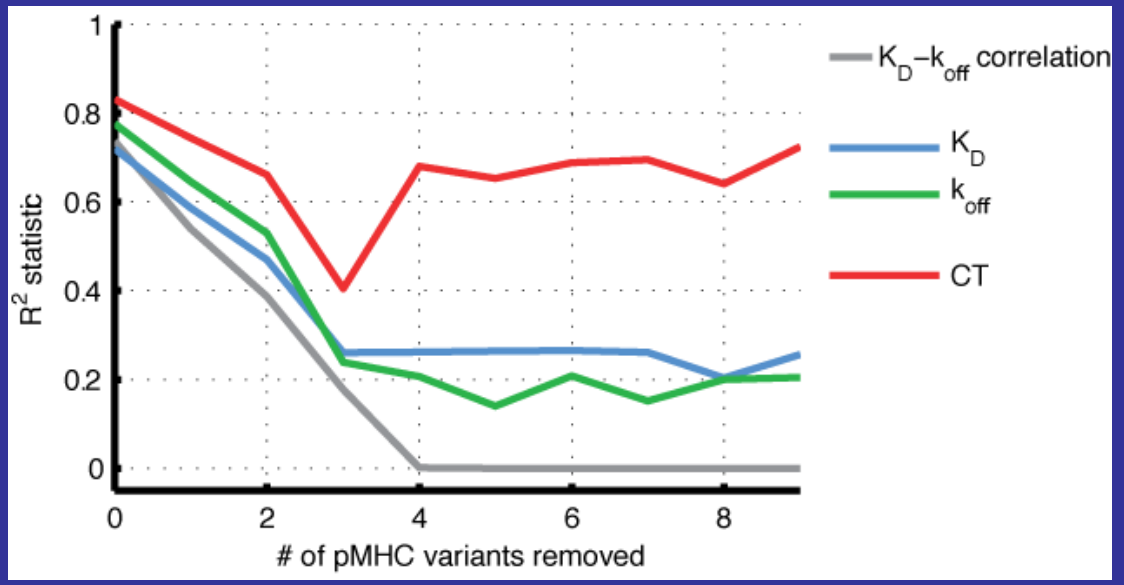
k^*_{on} (units of s^{-1}) is the intrinsic on-rate;
$k^*_{on} = \sigma\, k_{on}$
k_+ is the diffusion limited forward rate
k_- is the diffusive reverse rate

- if $k^*_{on} >> k_-$ then $k^*_{off} \sim K_D$
- if $k^*_{on} << k_-$ then $k^*_{off} \sim k_{off}$

Data fitting with the confinement time model



- improved correlation (R^2=0.83) compared to K_D and k_{off}.
- confinement time model R^2 statistic is more robust to removing data points.



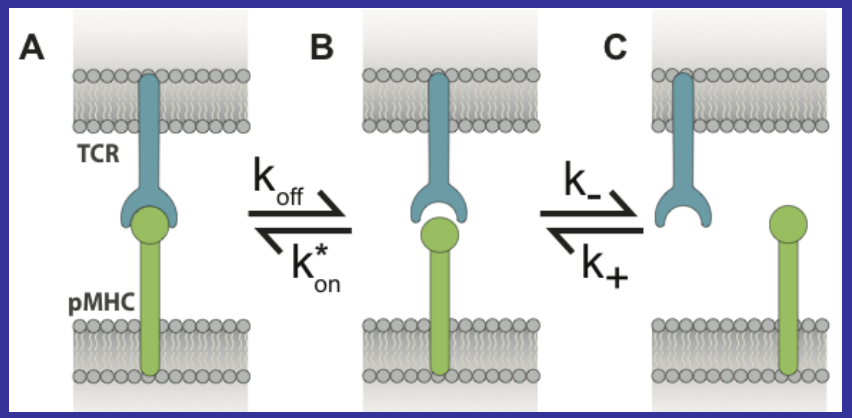
- the confinement time model is significant ($p<0.05$) for all except one data subset.

Comparison to previous studies

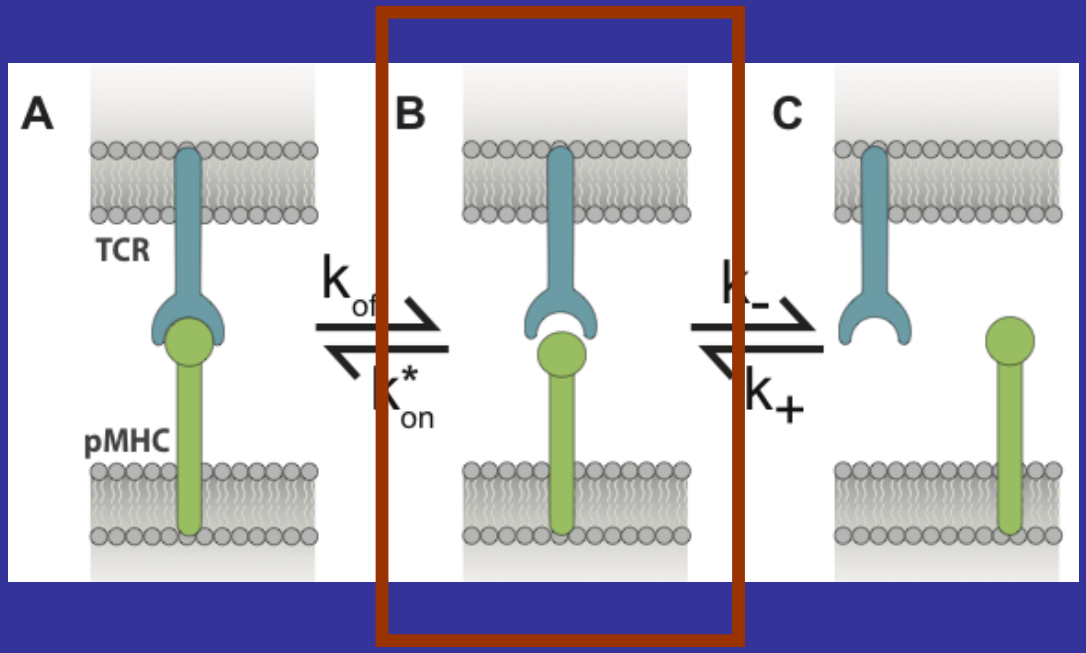
- Many previous studies are ~consistent with the confinement time model.
  - confinement time reduces to k_{off} or K_D under certain conditions.
- When k_{on} is large (>10^5 $M^{-1}s^{-1}$), pMHC potency correlates well with K_D (Holler and Kranz, 2003; Tian et al., 2007)
- With smaller k_{on} (~10^3 $M^{-1}s^{-1}$), potency correlates well with k_{off} (Krogsgaard et al., 2003).
- Our k_{on} values were intermediate (~10^4 $M^{-1}s^{-1}$).
  - This may explain why the confinement time model provided the best description of our data.

Evidence for the confinement time model

- Interactions at cell-cell interfaces last longer than solution measurement predicts. (Grakoui et al., 1999; Tolentino et al., 2008).
  - Lifetime of a CD2-CD58 bond is 100x longer than in solution.
  - However: membrane-tethered interactions could be shorter because of mechanical forces.
  - Direct measurements of 2D TCR/pMHC bond lifetimes will help!
- Increasing pMHC mobility on the cell surface inhibits T cell activation. (Luxembourg et al., 1998; Segura et al., 2008; Wettstein et al., 1991).
  - Confinement time model says that increased mobility decreases rebinding.

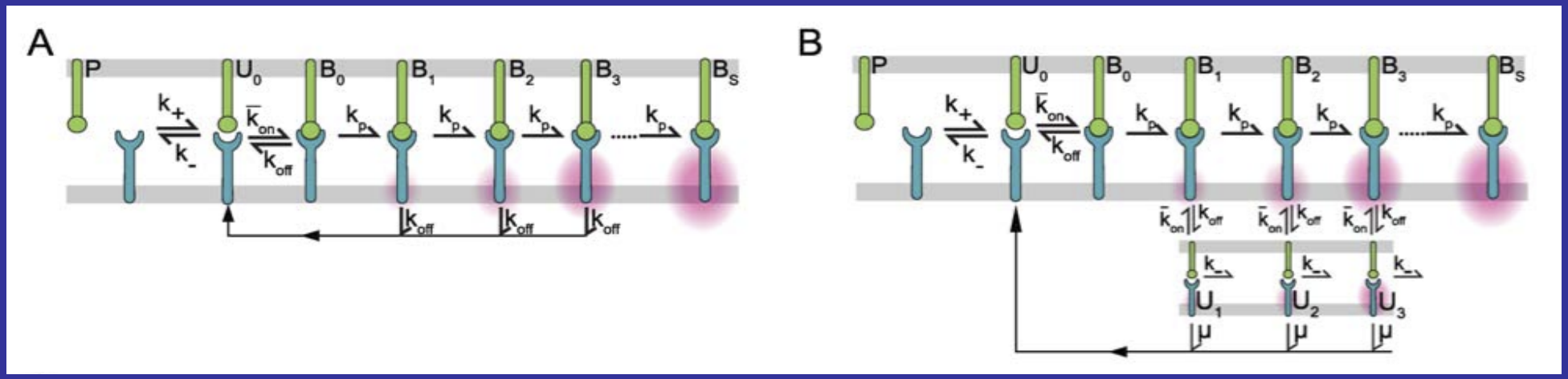


Confinement time model and signal persistence

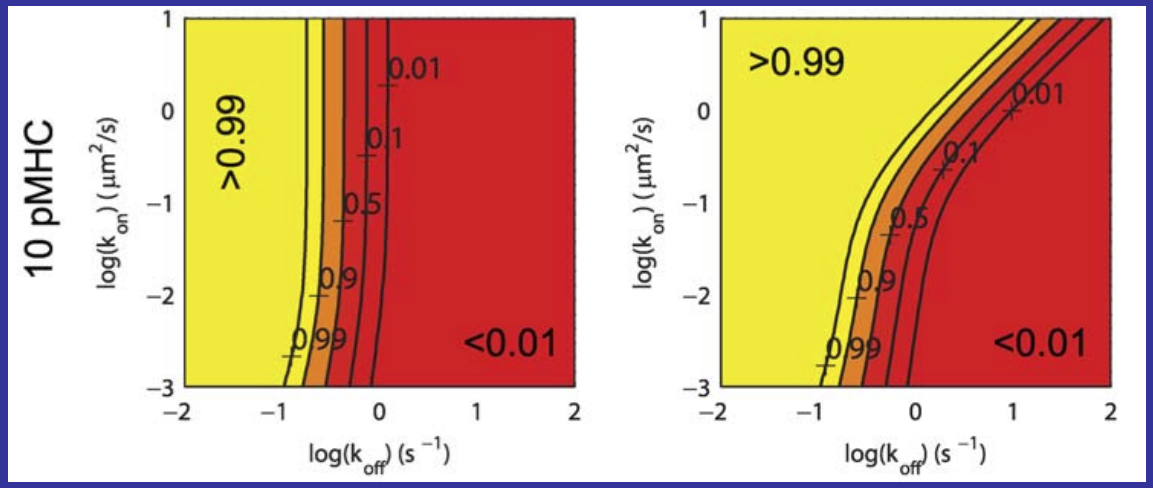


- What is the role of rebinding in antigen discrimination?
- Focus on short-time effects (first 30s of cell interaction).

A confinement model with signal persistence for **early** TCR signaling



- Modified kinetic proofreading allows **signals to persist** for a short time after pMHC unbinding.



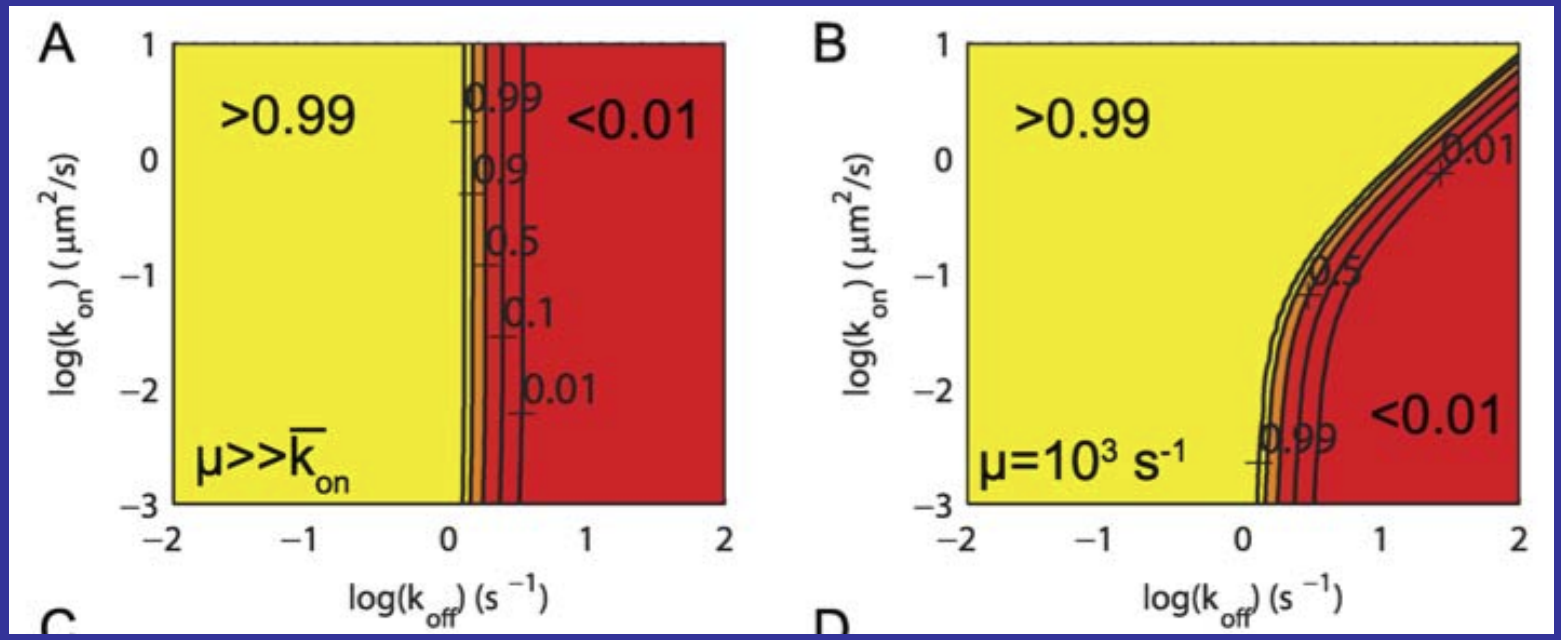
Probability of one productive signal after 30s interaction.

- We find an important role for k_{on} as well as k_{off} in signal discrimination.

Weak pMHC cannot conspire to signal in this model

(A) no signal persistence

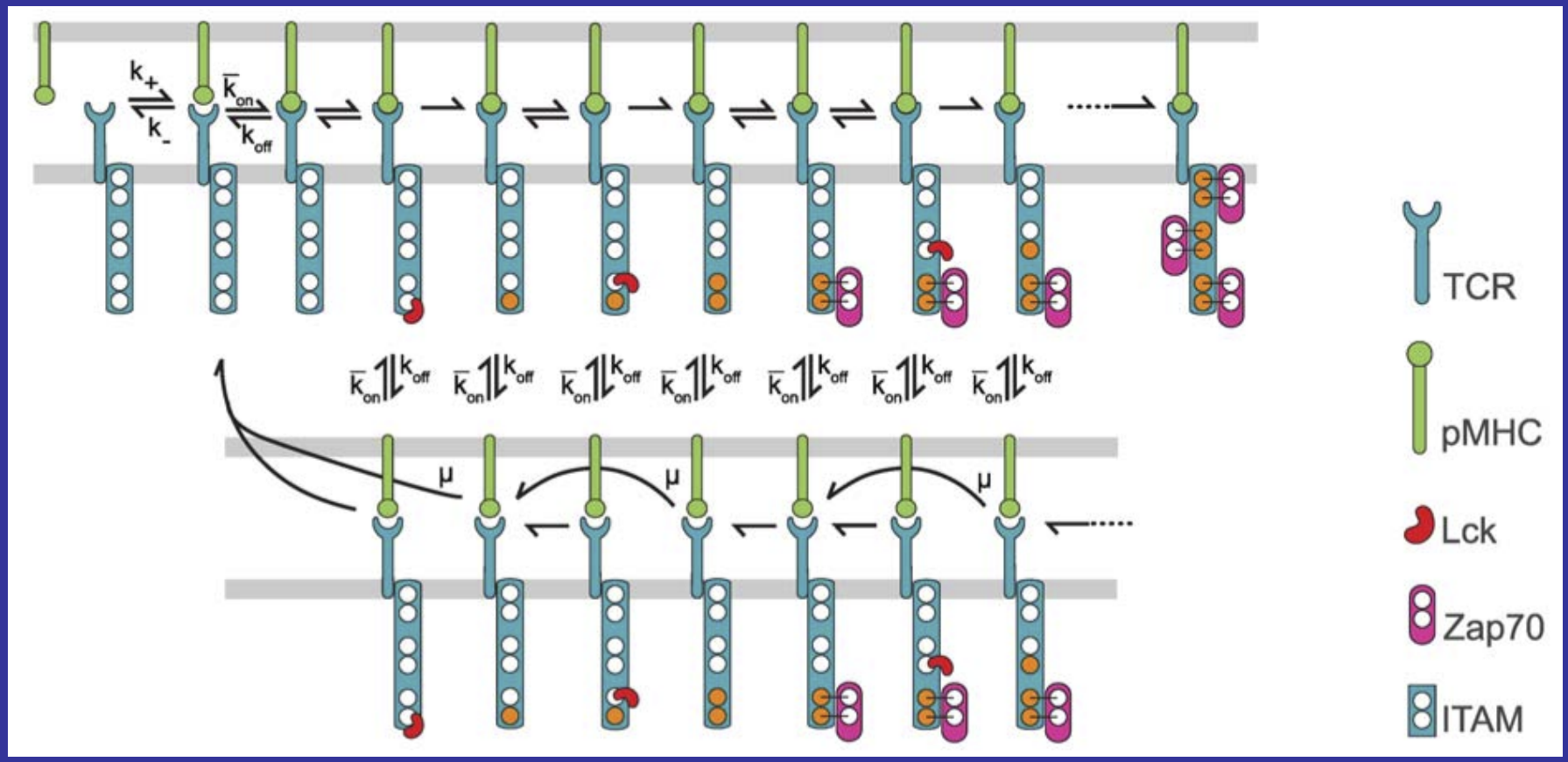
(B) 0.001s persistence



Contours: probability that at least 1 TCR out of 7854 at the contact interface signals within 30s. 39270 identical pMHC are present.

- For short persistence times, weak pMHC (low k_{on} / large k_{off}) do not signal.
- If signal persistence is long, a sequence of pMHC may activate a few TCR.

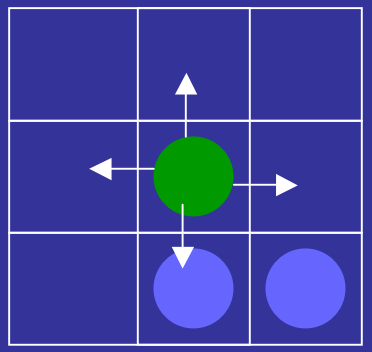
All these findings are recapitulated if we use a more detailed TCR signaling model.

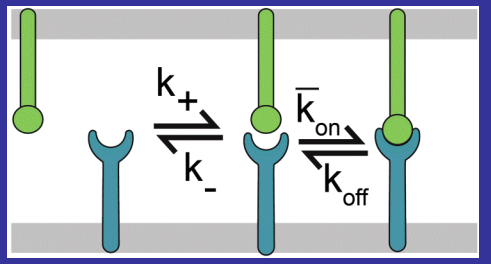


Does the ODE model accurately capture the diffusion effect?

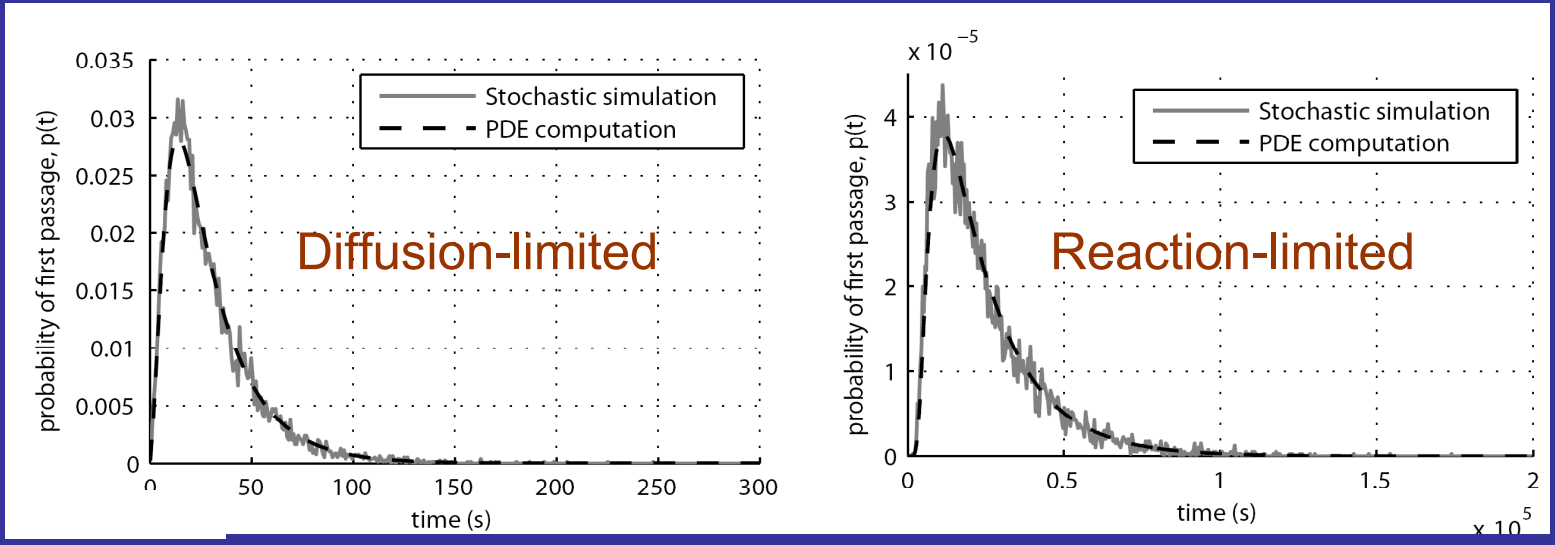
1. Formulate a discrete-space continuous-time simulation based on the Gillespie algorithm.
   - Single pMHC diffusing on an array of immobile TCR.

Based on the work of Isaacson and Peskin (2006) SIAM Sci. Comp. 28:47-74





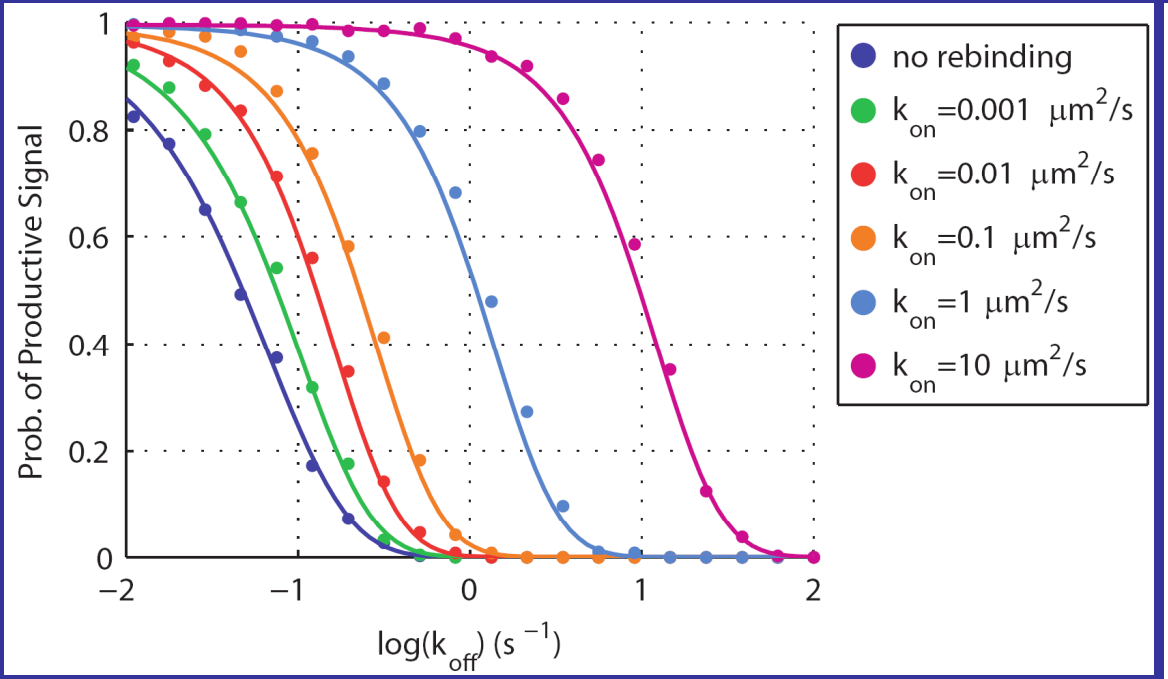
2. This microscopic spatial simulation agrees with macroscopic PDE.

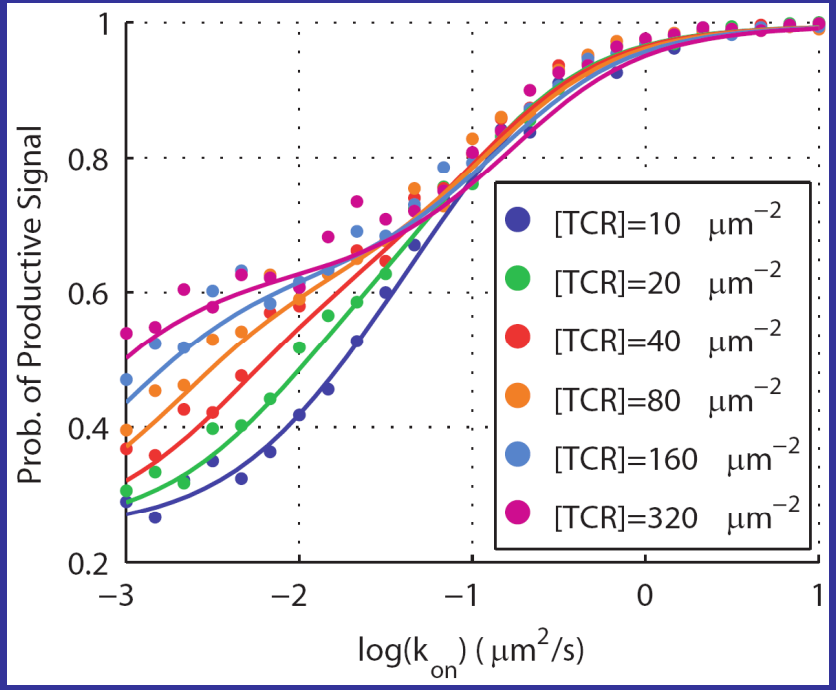


Therefore we have an accurate spatial simulation.

ODE model captures the effects of membrane diffusion.

3. Spatial simulations (dots) compare favorably to the ODE formulation (lines).

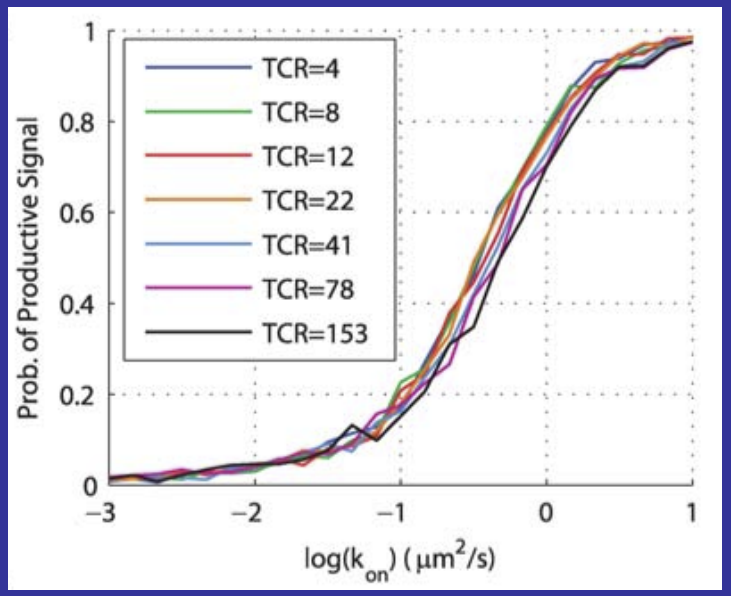


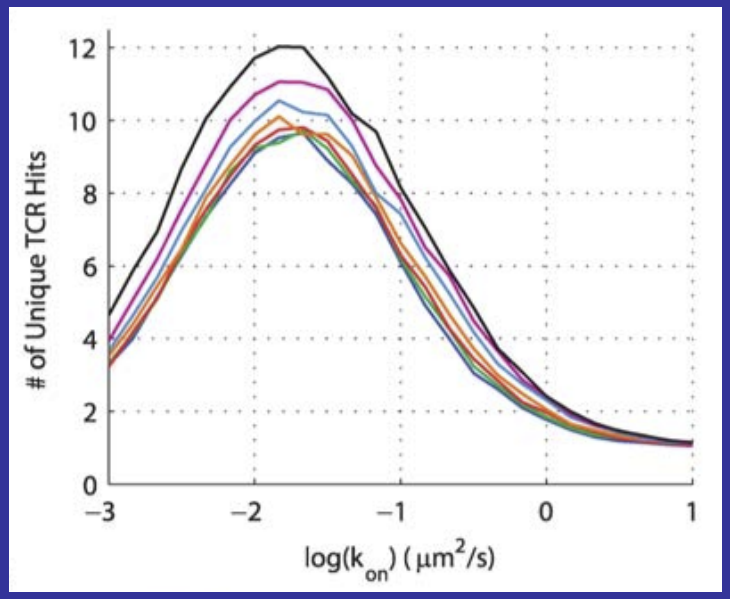


• the ODE formulation accurately captures the effects of membrane diffusion in the parameter regimes we are considering.

TCR clustering does not improve signaling

- TCR cluster size has a negligible effect on productive signaling after 30s
- TCR clustering
  - increases the number of unique TCR bound by a pMHC
  - reduces the probability of escaping the cluster





- Underlines the importance of pMHC rebinding to the same TCR versus serial binding of pMHC to different TCR.

[Spatial lattice Monte Carlo simulation of one pMHC diffusing and binding to TCR starting with one pMHC bound at the center of a TCR cluster (r=100nm). A homogeneous distribution of TCR is assumed outside the cluster. TCR independently perform stochastic kinetic proofreading with signal persistence. The simulation is terminated when t=30s or a productive signal is transduced.]

Summary

Experiment:

- Response of T cells to a panel of 17 pMHC variants.
- Models based on TCR/pMHC confinement time consistently outperform other models.

Modeling:

- Signal persistence supports rapid and reliable early time antigen discrimination/detection.
    - discrimination on the basis of k_{on} and k_{off}
    - weak pMHC cannot conspire to signal
- ODE findings are supported by explicit spatial model.
- Findings are robust to using a more detailed TCR signaling model.

- D. Coombs et al. Nature Immunology 3:926 (2002).
- P.A. Gonzalez et al. PNAS 102:4824 (2005).
- O. Dushek, R. Das and D. Coombs PLoS Comp Biol 5:e1000578 (2009).
- M. Aleksic, O. Dushek et al. Immunity, in press (2010).